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07/755,273	09/05/91	VAN DER LOAN	54563/GBRD-U

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EXAMINER: HENDRICKSON

ART UNIT	PAPER NUMBER
1814	11

DATE MAILED: 07/15/92

This is a communication from the examiner in charge of your application
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☒ Responsive to communication filed on 4-28-92 ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☐ Notice of References Cited by Examiner, PTO-892.
2. ☐ Notice re Patent Drawing, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 4-7, 9-17, 19, and 23-26 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☒ Claims 1-3, 8, 18, and 20-22 have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 4-7, 9-17, 19, and 23-26 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____; filed on _____.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1814.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to provide an adequate writt n description of the invention, and failing to adequately teach how to make and/or use the invention, i.e. failing to provide an enabling disclosure.

The specification describes the manipulation of the protease gene from strain PB92, but does not describe a way of obtaining such DNA, or a source of the DNA or strain.

Claims ~~4-17,~~ 4-9,9-17 19 and 23-26 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to th specification.

Claims 12, 14, 17, 23, 26, and thus their dep ndent claims ~~4-11,~~ 4-7,9-11 19, and 24-25 are rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited

to methods of producing an alkalophilic asporogenic Bacillus novo species PB92 of minimal indigenous extracellular protease level, transformed with a mutated B. novo PB92 alkaline protease. See M.P.E.P. §§ 706.03(n) and 706.03(z).

Claims 12, 14, 23 and 26 are not properly enabled for th recitation of "reduced extracellular alkaline protease levels", and "a gene coding for a high alkaline protease". The applicant's have not shown that this is possible, and actually part of the invention, for each and every extracellular/alkalin protease gene in each and every Bacillus species, as all are not known nor cloned. Further, the applicant has not shown this to be true for all protease molecules of the particular Bacillus novo species PB92 strain exemplified; only the protease in which the corresponding wild-type gene has been deleted from the Bacillus host are sufficiently expressed upon transformation with the mutated gene, without production of that particular "wild-type" protease.

The claims are not properly enabled for the recitation of the phrase "mutant high alkaline protease", and claim 17 is also not enabled for such proteases "differing in at least one amino acid from a wild-type high alkaline protease". One of ordinary skill in the art would not b able to determine what type of mutation, how many, at what amino acid, tc., including all variations possible in order to fulfill what the applicants truly regard as the invention.

The claims are not properly enabled for any or all "alkalophilic Bacillus" strains. There are several such strains known and yet unknown, ~~such as B. licheniformis, B.~~ delete amyliquefaciens, B. subtilis, B. novo species PB92, etc. One of ordinary skill would not be able to perform the invention with each alkaline protease from each type of "alkalophilic Bacillus" strain, if indeed they possess this gene to be deleted initially.

The claims are also not properly enabled for the term "reduced", regarding the extracellular alkaline protease level of the cell. This encompasses anywhere from 0-99 % of the original levels of protease activity. The deletion of the gene for a given protease should reduce the activity far below that of basal levels. This is not reflected in the claims.

Claims 2, 9, 12, 14, and 17-21 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 12 is indefinite, as it is not clear from the claim language where the gene for the indigenous protease is located within the cloning vector, or if th vector is merely "comprising the 5' and 3' flanking regions of a gene coding for" the protease, or if indeed it is to be deleted, and how much of a sequence is 1 ft.

Claim 14 is vague and indefinite for the recitation of the phrase "capable of producing", as it is not clear as to the definition of this, and how this phrase defines said species different from any other form of protein-producing strain.

Claim 17 is confusing as to which alkaline protease, the claimed intended protease or "a wild-type", is the one that "differs in at least one amino acid from the wild-type protease produced". Claim 18 improperly recites "said Bacillus strain" from claim 17, but lacks a clear antecedent basis for this in the independent claim (17). Claims 19-21 are confusing and/or incorrect in the recitation of "proteases according to claim 16", as claim 16 is directed to a Bacillus strain, and does not discuss any protease, let alone more than one. Claims 20-21 are of improper format, for the recitation of "Use of one...". It is suggested that these claims be written using acceptable claim language, thus setting forth positive method steps.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if th differences between the subj ct matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the inv ntion was made to a p rson having ordinary skill in th art to which said subject matter pertains. Patentability shall not be negatived by th mann r in which the invention was made.

> Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims ~~4-17~~ 4-7, 9-17, 19, and 23-26 are rejected under 35 U.S.C. § 103 as being unpatentable over Fahnestock et al. and Estell et al., in view of TeNijenhuis and Suggs et al.

Fahnestock et al. disclose that "Bacillus strains having reduced levels of extracellular protease are produced by replacing the native chromosomal DNA sequence comprising the gene for an extracellular protease, such as subtilisin, with a partially homologous DNA sequence having an inactivating DNA segment inserted therein." The alkaline protease gene is inactivated by inserting a DNA fragment of chloramphenicol acetyltransferase (CAT) into the protease gene. Using homologous recombination, the original, functional gene is deleted. Also, the replication function of the cloning vector is inactivated, yielding a reduced-protease strain. "While subtilisin is the most abundantly produced Bacillus exoprotease, it will be readily appreciated that the procedures described herein can also be employed to inactivat other exoprotease gen s in th Bacillus chromosomal DNA, ther by producing strains having ven furth r reduced extrac llular protease levels. [column 4]" Th se strains w re also produced to b neutral protease n gative. The resultant "strains carrying the inactivated apr gene are

excellent candidates for use as hosts for the expression and secretion of heterologous genes".

Estell et al. disclose a similar process for the expression of proteins in Bacillus strains, doing so by deleting the extracellular protease genes, and inserting the mutated alkaline protease gene of choice for expression (see columns 7-8).

TeNijenhuis discloses a purified "high" alkaline protease from Bacillus nov. sp. PB92.

Suggs et al. teach the use of mixtures of chemically synthesized oligodeoxyribonucleotides as hybridization probes for the isolation of specific cloned DNA sequences. The approach is to "chemically synthesize a mixture of oligonucleotides that represent all possible codon combinations for a small portion of the amino acid sequence of a given protein." Once a protein, in this case the alkaline protease, is purified, amino acid sequencing can be performed by any of the techniques well known and used in the art, such as the method of modern automated Edman degradation. Under the principle that one sequence must be complementary to the DNA for that protein, "the complementary oligonucleotide will form a perf ctly base paired dupl x with the DNA from the coding region...". Thus, mixed oligonucleotide probes allow the isolation of DNA sequences for any protein with a known portion of the amino acid sequence.

In light of the method of Suggs et al. for isolating the appropriate DNA sequence coding for a particular protein, it would have been obvious to one of ordinary skill in the art to use these methods to determine the coding nucleotide sequence of alkaline protease in the organism Bacillus PB92. TeNijenhuis discloses the fact that Bacillus PB92 produces this alkaline protease and therefore possesses that gene. One would not have to probe a library of possible sources to find a similar gene, as the references provide sufficient motivation to merely determine the sequence from the known source.

Thus, the pure production of alkaline proteases, or of the strain PB92 "high" alkaline protease of claims 4-5, 9, 13 and 15, without other interfering proteases, would have been well within the ordinary level of skill in the art to do, and obvious to perform given the teachings of the instant references. Estell et al. and Fahnestock et al. disclose the instant method for any heterologous protein, particularly alkaline proteases, and thus the incorporation of the gene for the enzyme of TeNijenhuis into this method would have been an obvious step.

Finally, the limitation of "an alkalophilic Bacillus strain" does not render the claim patentably distinct from th similar methods of Fahnestock et al. and Estell et al., per se. The systems are the same, and both used with Bacillus organisms, which ar already alkalophilic. Further, the mutation of the strain to produce an "asporogenic" variant is obvious and well

known in the art to do, and is easily obtained via classic UV mutation techniques. Thus, the claims are not deemed patentable in view of the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Keith Hendricks whose telephone number is (703) 308-0452.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

KDH
kdh
July 6, 1992

ROBERT A. WAX
SUPERVISORY PATENT EXAMINER
GROUP 180